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Three New Species of *Dicyema* (Dicyemida: Dicyemidae) from *Octopus kagoshimensis* (Mollusca: Cephalopoda: Octopodidae)

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Three new species of dicyemid mesozoan are described from *Octopus kagoshimensis* Ortmann, 1888 collected off Irino in Tosa Bay, sourthern Shikoku, Japan. *Dicyema tosaense* sp. nov. is a small species that reaches about 800 μm in length. The vermiform stages are characterized by 16–18 peripheral cells, a conical calotte, and an axial cell that extends to the base of the metapolar cells. Infusoriform embryos consist of 37 cells; two nuclei are present in each urn cell and the refringent bodies are solid. *Dicyema irinoense* sp. nov. is a species of medium size that reaches about 1300 μm in length. The vermiform stages are characterized by 22 peripheral cells, a disc-shaped calotte, and an axial cell that extends to the base of the propolar cells. Infusoriform embryos consist of 37 cells; a single nucleus is present in each urn cell and the refringent bodies are solid. *Dicyema sphaerocephalum* sp. nov. is a species of medium size and reaches about 1000 μm in length. The vermiform stages are characterized by 18–20 peripheral cells, a rounded calotte, and an axial cell that extends to the base of the propolar cells. Infusoriform embryos consist of 37 cells; two nuclei are present in each urn cell and the refringent bodies are solid. This is the first report of dicyemids in the octopus *O. kagoshimensis*.
Key Words: cephalopods, dicyemids, infusoriform embryos, mesozoans, parasites, renal organs, *Octopus kagoshimensis*, vermiform embryos.

Introduction

The first record of dicyemids in Japan was published by Nouvel and Nakao (1938). They described *Dicyema misakiense* Nouvel and Nakao, 1938 from *Octopus vulgaris* Lamarck, 1798, and *D. orientale* Nouvel and Nakao, 1938 from *Sepioteuthis lessoniana* Lesson, 1830. Nouvel (1947) later described *D. acuticephalum* Nouvel, 1947 from *O. vulgaris* and identified a dicyemid species from *Sepia esculenta* Hoyle, 1885 as *Pseudicyema truncatum* Whitman, 1883, which had been described earlier in Europe. Subsequently, two dicyemid species, *D. japonicum* Furuya and Tsuneki, 1992 and *D. clavatum* Furuya and Koshida, 1992, were described from *O. vulgaris* and *O. minor* (Sasaki, 1920) (currently *Callistoctopus minor*), respectively (Furuya

et al. 1992a). Furuya (1999) also reported 14 new species of dicyemid from six cephalopod species caught off the coasts of Japan: *Octopus fangsiao* d'Orbigny, 1840, *O. minor* (currently *Callistoctopus minor*), *O. hongkongensis* Hoyle, 1885, *O. dofleini* (Wülker, 1910) (currently *Enteroctopus dofleini*), *Sepia esculenta*, and *S. lycidas* Gray, 1849. Furuya and Tsuneki (2003) recorded two undescribed species of dicyemid from *Octopus areolatus* (de Haan, 1840) from Japan. More recently, a new dicyemid was described from *Sepioteuthis lessoniana* by Furuya and Tsuneki (2005).

In this paper three new species in the genus *Dicyema* are described from *Octopus kagoshimensis* Ortmann, 1888 collected off Irino in Tosa Bay, Shikoku. These are the first dicyemids to be described from *O. kagoshimensis*.

Materials and Methods

Twenty-one individuals of *Octopus kagoshimensis* obtained from fishermen were examined for dicyemids from May, 2000, to April, 2001. The size, sex, and maturity of each octopus are indicated in Table 1.

Table 1. *Dicyema* species from the octopus *Octopus kagoshimensis* collected off Irino in Tosa Bay, Shikoku, Japan.

Host No.	ML[1] (mm)	Sex	Maturity stage[2]	Date of examination	*Dicyema* species
KA563	60	♂	M	26 May 2000	*D. tosaense*
KA564	47	♂	M	26 May 2000	*D. tosaense*+*D. irinoense*
KA565[a]	55	♀	M	26 May 2000	*D. tosaense*+*D. irinoense*
KA566	46	♂	M	26 May 2000	*D. tosaense*
KA567	46	♂	M	26 May 2000	*D. tosaense*
KA568	38	♂	S	26 May 2000	*D. tosaense*
KA569	47	♂	M	26 May 2000	*D. tosaense*
KA570	58	♂	M	26 May 2000	*D. tosaense*
KA685	67	♀	M	07 Mar. 2001	*D. tosaense*
KA686[b]	52	♂	M	07 Mar. 2001	*D. tosaense*+*D. sphaerocephalum*
KA719	40	♂	M	02 Apr. 2001	*D. tosaense*
KA721	39	♂	M	02 Apr. 2001	*D. tosaense*+*D. irinoense* +*D. sphaerocephalum*
KA722	53	♂	M	02 Apr. 2001	None
KA752	35	♀	S	03 Apr. 2001	*D. sphaerocephalum*
KA753	43	♂	M	03 Apr. 2001	None
KA754	46	♀	M	03 Apr. 2001	None
KA755	50	♀	M	03 Apr. 2001	*D. tosaense*
KA756	57	♂	M	03 Apr. 2001	*D. tosaense*+*D. sphaerocephalum*
KA757	56	♂	M	03 Apr. 2001	*D. tosaense*
KA758	51	♂	M	03 Apr. 2001	*D. tosaense*+*D. sphaerocephalum*
KA759	58	♂	M	03 Apr. 2001	*D. sphaerocephalum*

[a] The host (symbiotype) for the syntypes of *D. tosaense* and *D. irinoense*.
[b] The host (symbiotype) for the syntypes of *D. sphaerocephalum*.
[1] ML, dorsal mantle length.
[2] M, mature; S, submature.

When dicyemids were detected in the kidney of a host cephalopod, small pieces of renal appendages with attached dicyemids were removed and smeared on glass slides. The smears were fixed immediately in Bouin's fluid for 24 hr and then stored in 70% ethyl alcohol. Most slides were stained in Ehrlich's hematoxylin and counterstained in eosin. Stained smears were mounted using Entellan (Merck). Dicyemids were observed with a light microscope (Olympus BH-2) at magnifications up to 2000×. Measurements and drawings, respectively, were made with the aid of an ocular micrometer and a drawing tube (Olympus U-DA).

Nouvel (1948), Short and Damian (1966), Furuya *et al.* (1992b), and Furuya (1999) give the terminology for cell names used in the description of infusoriform larvae.

Syntypes of the dicyemids are deposited in the Osaka University Museum, Toyonaka, Osaka, Japan (OUM), the Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH), and in the author's collection. The host octopuses of the syntypes of the three new dicyemid species are deposited in the OUM.

Abbreviations for Figs: A, apical cell; AG, agamete (axoblast); AI, apical internal cell; AL, anterior lateral cell; AX, axial cell; C, couvercle cell; CA, capsule cell; CL, calotte; D, diapolar cell; DC, dorsal caudal cell; DE, developing infusoriform embryo; DI, dorsal internal cell; DV, developing vermiform embryo; E, enveloping cell; G, germinal cell; I, infusoriform embryo; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; NI, nucleus of axial cell of infusorigen; O, oogonium; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PO, primary oocyte; PVL, posteroventral lateral cell; R, refringent body; S, spermatogonium; SP, sperm; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell; V3, third ventral cell.

Taxonomy

Dicyema tosaense sp. nov.

(Figs 1, 2, Tables 1, 2)

Diagnosis. Small-sized dicyemids, body lengths typically exceeding 800 μm. Peripheral cell numbers of vermiform stages (i.e., vermiform embryo, nematogen, and rhombogen) 16–18: 4 propolars, 4 metapolars, and 8–10 trunk cells. Calotte relatively large, conical. Infusoriform embryos consisting of 37 cells; urn cells with 2 nuclei each.

Description. *Nematogens* (Fig. 2a, c). Body slender; lengths ranging from 500 to 800 μm, widths from 30 to 60 μm. Peripheral cell numbers 16–18: 4 propolars, 4 metapolars, 2 parapolars, 4–6 diapolars, and 2 uropolars. Calotte bluntly rounded, conical (Fig. 1a, b). Cilia on calotte short, about 6 μm long, oriented forwards. Cytoplasm of both propolar and metapolar cells stained by hematoxylin (Fig. 1a). Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively. Trunk mostly uniform in width; trunk cells arranged in opposed pairs. Axial cell cylindrical, rounded anteriorly, extending forward to base of metapolar cells (Fig. 2a–c). In axial cell of large individuals, 12–15 vermiform embryos present. Accessory nuclei seen in peripheral trunk cells.

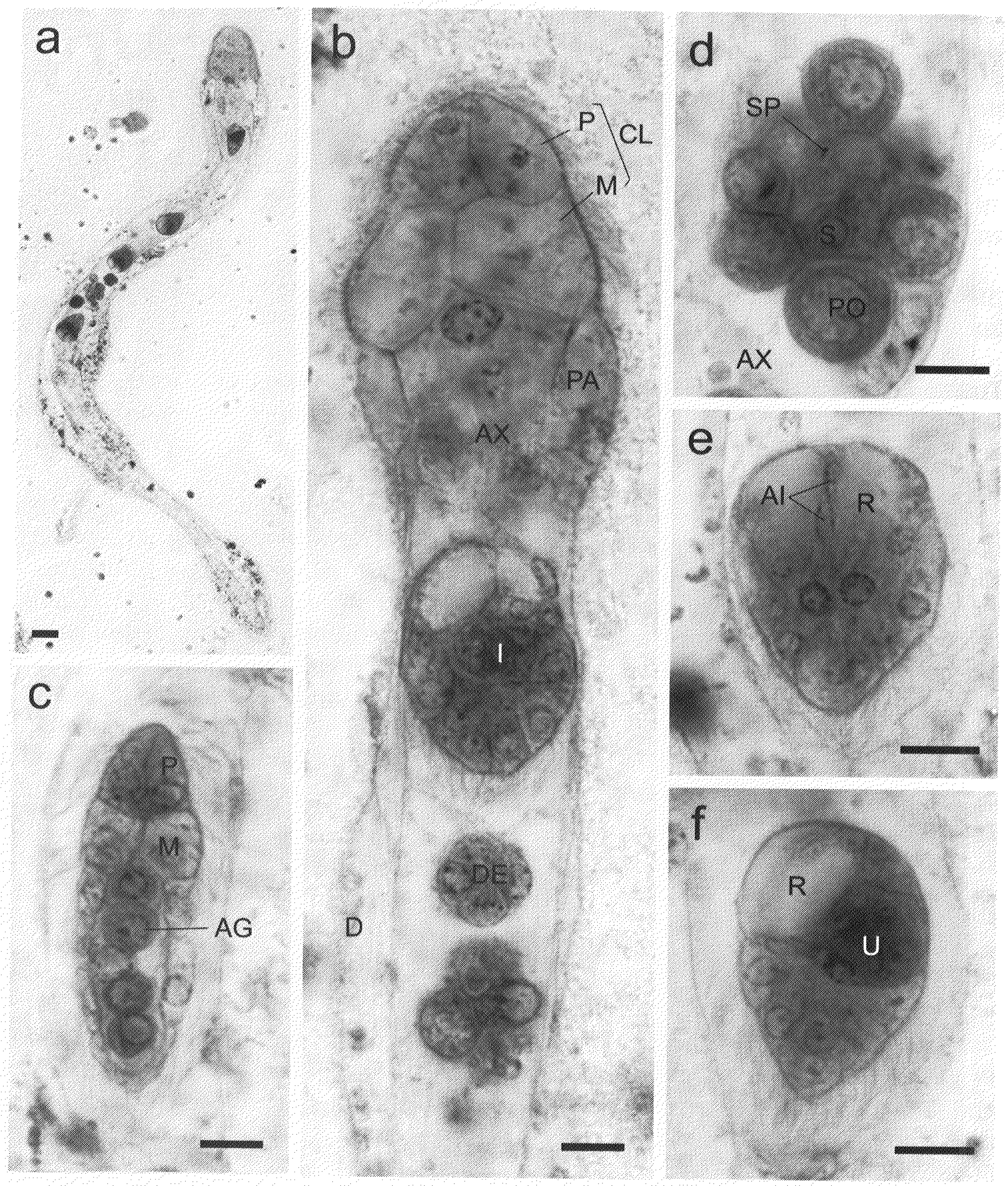

Fig. 1. *Dicyema tosaense* sp. nov., syntype specimens on slide OUM-ME-00003. a, Rhombogen, entire; b, rhombogen, anterior region; c, vermiform embryo within axial cell; d, infusorigen; e–f, infusoriform embryos, horizontal section (e) and sagittal section (f). Scale bars: a=20 μm, b–f=5 μm. Abbreviations as in "Materials and Methods".

Vermiform embryos (Figs 1c, 2d, e). Full-grown vermiform embryos small-sized; lengths ranging from 20 to 40 μm, widths from 8 to 12 μm; peripheral cell numbers 16–18 (Table 2). Anterior end of calotte tapered anteriorly, bluntly rounded at tip. Trunk cells arranged in opposed pairs. Axial cell rounded anteri-

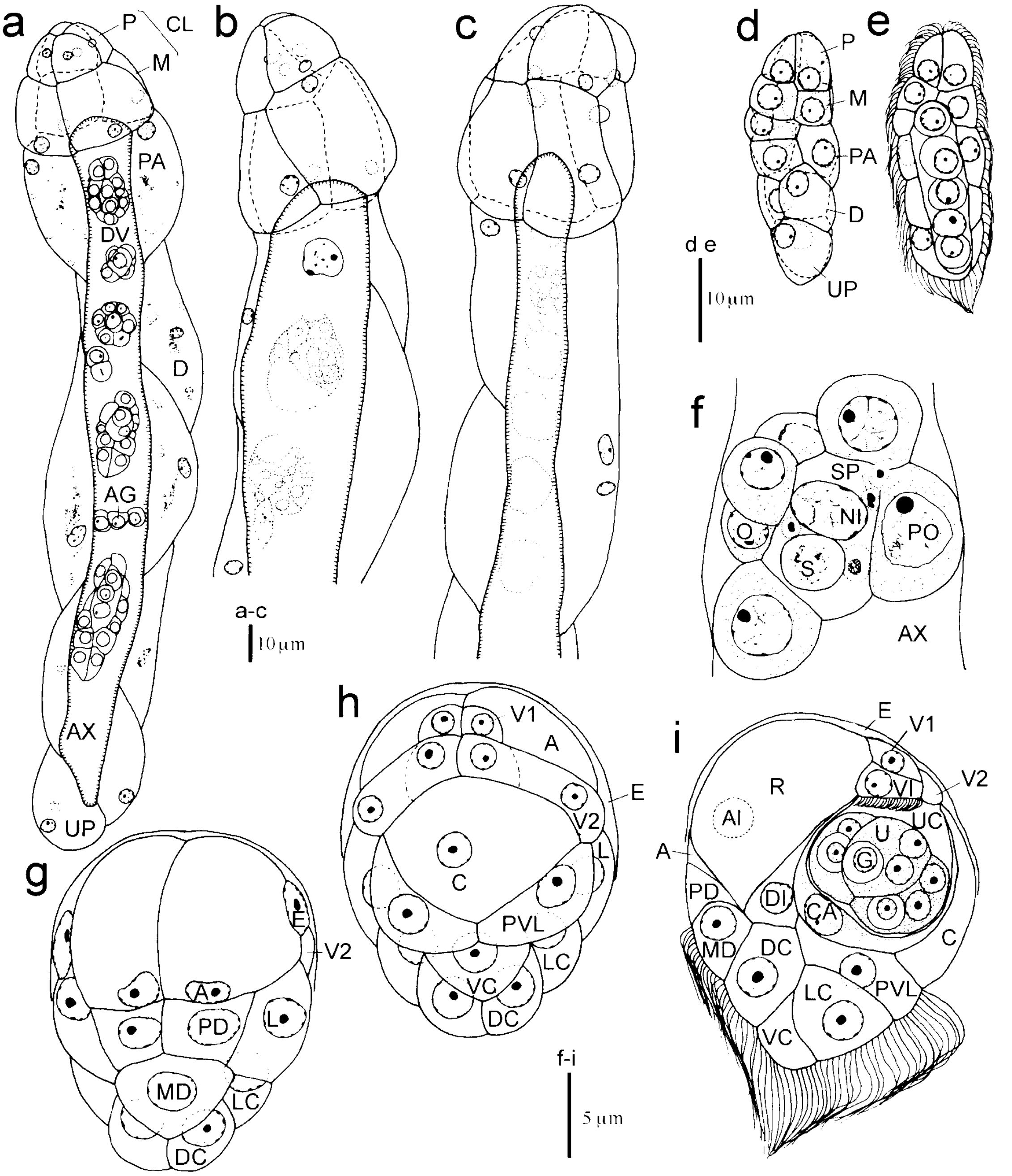

Fig. 2. *Dicyema tosaense* sp. nov., syntype specimens on slide OUM-ME-00003. a, Nematogen, entire; b, rhombogen, anterior region; c, nematogen, anterior region; d–e, vermiform embryos within axial cell, cilia omitted (d) and optical section (e); f, infusorigen; g–i, infusoriform embryos, dorsal view (g; cilia omitted), ventral view (h; cilia omitted), and sagittal section (i). Abbreviations as in “Materials and Methods”.

orly, extending forward to base of metapolar cells. Axial cell nucleus typically located in center of axial cell. Axial cell of full-grown embryos containing 4–9 agametes.

Rhombogens (Figs 1a, b, 2b). Slightly stockier than nematogens, otherwise gen-

erally similar in shape and body proportions; lengths ranging from 500 to 800 μm, widths from 40 to 60 μm. Peripheral cell numbers 16–18 (Table 2). Calotte conical as in nematogens. Shape and anterior extent of axial cell similar to those of nematogens. Number of infusorigens present in axial cell 1 or 2; in axial cell of large individuals, 10–15 infusoriform embryos typically present. Accessory nuclei occasionally present in peripheral cells.

Infusorigens (Figs 1d, 2f). Small-sized. Axial cell of infusorigens usually rounded, diameter 6–8 μm. In mature infusorigens (n=20), number of external cells (oogonia and primary oocytes) 3–12 (mode, 6), number of internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes) 2–6 (mode, 3), and number of sperm 4–11 (mode, 7). Diameter of fertilized eggs 8.2 μm; diameter of sperm 1.3 μm.

Infusoriform embryos (Figs 1e, f, 2g–i). Ovoid, bluntly pointed posteriorly. In full-grown embryos (n=50), length (excluding cilia) 21.5±2.2 μm (mean±S.D.), length-width-height ratio 1: 0.77: 0.76. Cilia at posterior end 3.8 μm long. Refringent bodies present, solid, occupying anterior 50% of embryo length when viewed laterally (Fig. 2i). Nuclei of apical internal cells usually visible between refringent bodies (Fig. 1e). Cilia projecting from ventral internal cells into urn cavity (Fig. 2i). Cytoplasm of dorsal internal cells transparent. Capsule cells with many minute granular inclusions. Full-grown infusoriform embryos (n=50) consisting of 37 cells: 33 somatic and 4 germinal cells. Somatic cells of several types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, and 2 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); external cells without cilia (2 first ventral cells, 2 second ventral cells, and 1 couvercle cell); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 apical internal cells, 2 dorsal internal cells, 2 capsule cells, and 4 urn cells). Each urn cell containing 1 germinal cell plus 2 nuclei (Fig. 2i). Nuclei of second ventral cells pycnotic. All somatic nuclei typically becoming pycnotic as infusoriform embryos mature.

Type series. Syntypes: OUM-ME-00003 (1 slide, OUM); SBMNH-358800 (1 slide,

Table 2. Number of peripheral cells in three species of *Dicyema*.

Species	Cell number	Number of individuals		
		Nematogens	Vermiform embryos	Rhombogens
D. tosaense	21	0	0	1
	22	38	50	24
D. irinoense	18	2	4	1
	19	0	1	1
	20	23	31	14
D. sphaerocephalum	16	23	24	18
	17	11	16	15
	18	12	10	12

SBMNH); No. HF-KA565 (5 slides, author's collection).

Type locality. Japan, Shikoku, Kochi Prefecture, Tosa Bay, off Irino, 32°55′N, 137°10′E.

Host (symbiotype). *Octopus kagoshimensis* (Cephalopoda: Octopoda: Octopodidae), mature female, 55 mm ML (mantle length), OUM-MO-00003 for KA565.

Site of infection. Within renal sacs; anterior ends (calottes) inserted into crypts of renal appendages.

Incidence. Dicyemids found in 16 host cephalopods obtained in Tosa Bay (Irino), with 76.2% incidence among 21 cephalopods examined (see Table 1).

Distribution. Known only from the type locality.

Etymology. The specific name refers to Tosa Bay, the type locality.

Remarks. *Dicyema tosaense* is similar to *D. acuticephalum*, *D. bilobum* Couch and Short, 1964, *D. knoxi* Short, 1971, *D. typus* van Beneden, 1876, and *D. typoides* Short, 1964 in both calotte shape and number of peripheral cells (cf. van Beneden 1876; Couch and Short 1964; Short 1964, 1971). The infusoriform embryos of *D. tosaense* consist of 37 cells and are thus easily distinguished from those of *D. typus* (35 cells: Furuya *et al.* 2004) and *D. typoides* (also 35 cells: Furuya *et al.* 2004). The infusoriform embryos of *D. bilobum* and *D. knoxi* consist of 37 cells, but *D. tosaense* differs from these two species in the cellular composition of the infusoriform embryos: the infusoriform embryo of *D. bilobum* has third ventral cells (Furuya *et al.* 2004) and that of *D. knoxi* has postcapsular cells instead of apical internal cells (Short 1971).

In cellular composition and cell number of infusoriform embryos, *D. tosaense* is similar to *D. acuticephalum* (Furuya *et al.* 1997; Furuya, Hochberg *et al.* 2004); however, the infusoriform embryos are larger in *D. acuticephalum* than in *D. tosaense* ($29.8 \pm 2.2\,\mu$m vs. $21.5 \pm 2.2\,\mu$m). In addition, the axial cell of full-grown vermiform embryos of *D. tosaense* contains four to nine agametes whereas that of *D. acuticephalum* contains at most four agametes.

Dicyema irinoense sp. nov.

(Figs 3, 4, Tables 1, 2)

Diagnosis. Medium-sized dicyemids, body lengths typically not exceeding 1300 μm. Peripheral cell numbers of vermiform stages (i.e., vermiform embryo, nematogen, and rhombogen) 22: 4 propolars, 4 metapolars, 2 parapolars, and 12 trunk cells. Calotte disc-shaped; cephalic enlargement formed together with parapolar cells. Infusoriform embryos consisting of 37 cells; urn cells with single nucleus each.

Description. *Nematogens* (Figs 3b, 4a, c). Body hammer-like; lengths ranging from 500 to 1000 μm, widths from 30 to 60 μm. Peripheral cell numbers 22: 4 propolars, 4 metapolars, 2 parapolars, 10 diapolars, and 2 uropolars. Calotte disc-shaped in large individuals (Fig 3b), sometimes conical in small- to medium-sized individuals (Fig. 4c). Cilia on calotte about 4 μm long, oriented forwards. Cytoplasm of propolar and metapolar cells conspicuously stained by hematoxylin (Fig. 3a–c). Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively. Axial cell cylindrical, rounded anteriorly, extending forward to base of propolar cells (Fig 3b). About 10 vermiform embryos typically present in axial cell of large individuals.

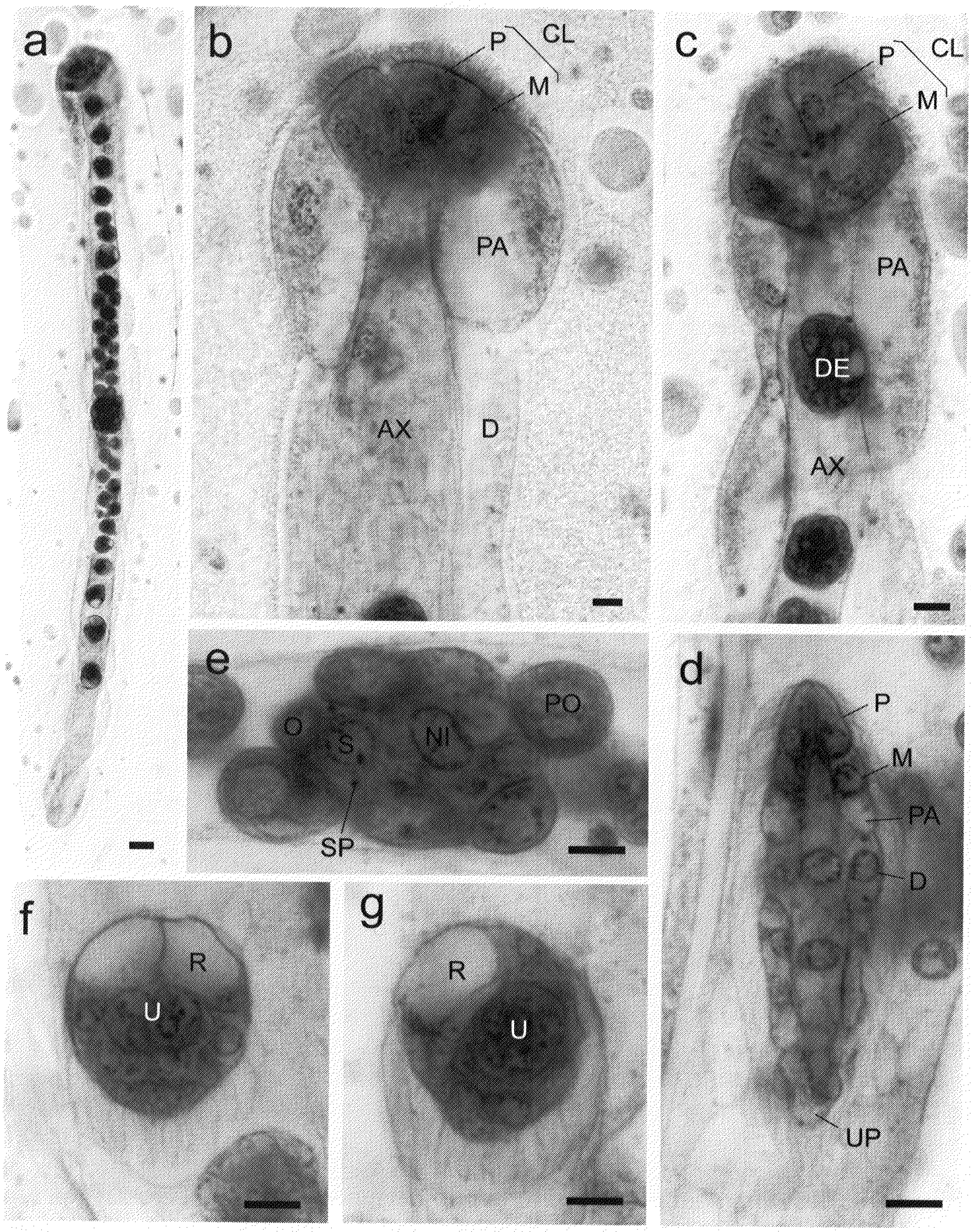

Fig. 3. *Dicyema irinoense* sp. nov., syntype specimens on slide OUM-ME-00004. a, Rhombogen, entire; b, nematogen, anterior region; c, young rhombogen, anterior region; d, vermiform embryo within axial cell; e, infusorigen; f–g, infusoriform embryo, horizontal section (f) and sagittal section (g). Scale bars: a=20 μm, b–g=5 μm. Abbreviations as in "Materials and Methods".

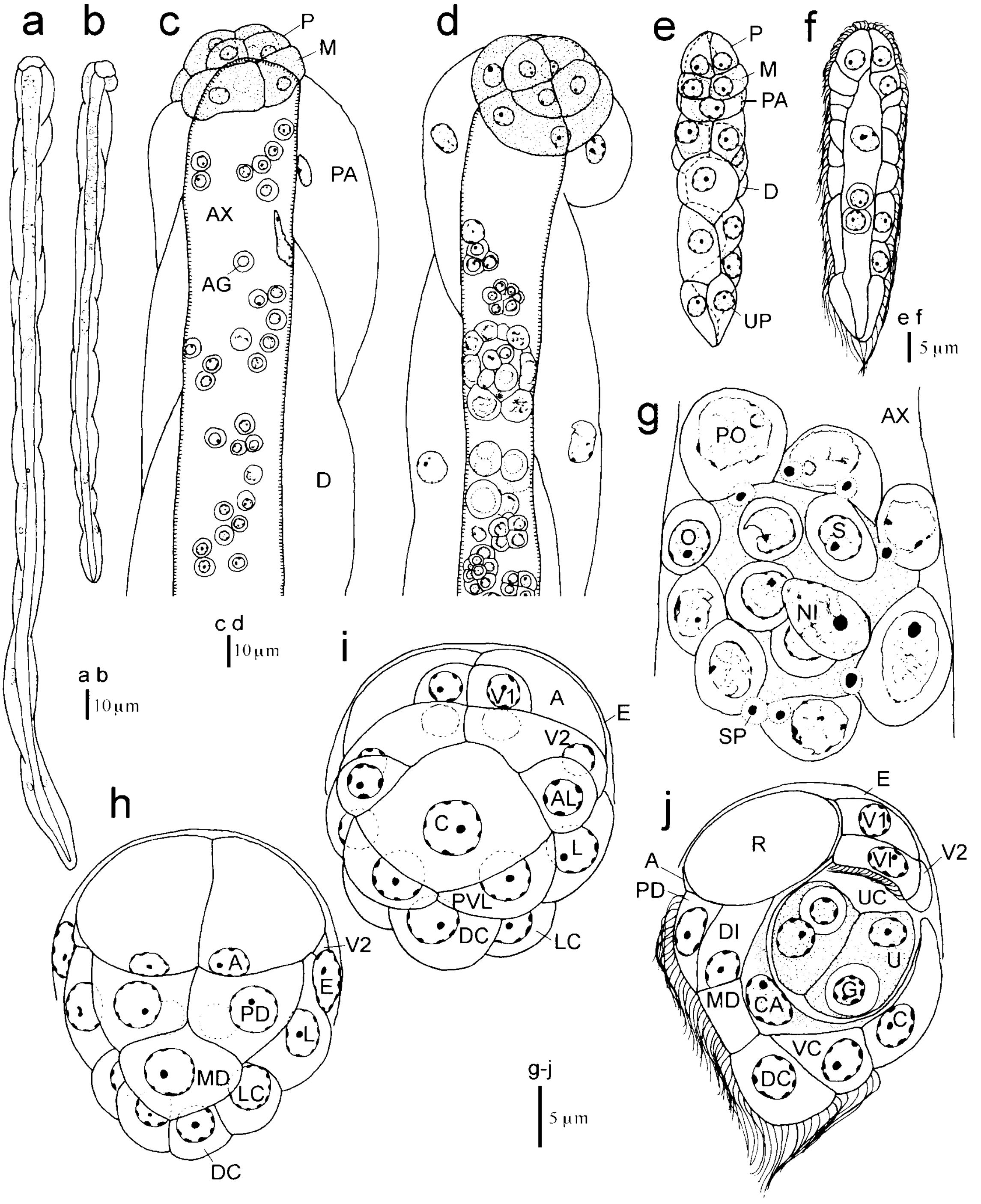

Fig. 4. *Dicyema irinoense* sp. nov., syntype specimens on slide OUM-ME-00004. a, Nematogen, entire; b, rhombogen, entire; c, nematogen, anterior region; d, rhombogen, anterior region; e–f, vermiform embryos within axial cell, cilia omitted (e) and optical section (f); g, infusorigen; h–j, infusoriform embryos, dorsal view (h; cilia omitted), ventral view (i; cilia omitted), and sagittal section (j). Abbreviations as in "Materials and Methods".

Vermiform embryos (Figs 3d, 4e, f). Full-grown vermiform embryos small-sized; lengths ranging from 30 to 50 μm, widths from 9 to 12 μm; peripheral cell number 22 (Table 2). Anterior end of calotte rounded. Trunk cells arranged in opposed pairs. Axial cell tapered anteriorly, extending forward to base of propolar cells, as in nematogens. Axial cell nucleus usually located in center or occasionally in anterior half of axial cell. Axial cell of full-grown embryos typically containing 1 or 2 agametes.

Rhombogens (Figs 3a, c, 4b, d). Slightly stockier than nematogens, otherwise generally similar in shape and body proportions; lengths ranging from 1000 to 5000 μm, widths from 30 to 60 μm. Peripheral cell number usually 22, occasionally 20 (Table 2). Cephalic enlargement composed of calotte and parapolar cells as in nematogens. Calotte disc-shaped in large individuals (Fig 4d), sometimes conical in small- to medium-sized individuals (Fig. 3c). Shape and anterior extent of axial cell similar to those of nematogens. Number of infusorigens present in axial cell of rhombogen ranging from 1 to 3; in axial cell of large individuals, 10–40 infusoriform embryos typically present. Uropolar cells verruciform. Accessory nuclei occasionally observed in trunk peripheral cells.

Infusorigens (Figs 3e, 4g). Medium-sized. Axial cell of infusorigens usually irregular in shape. In mature infusorigens (n=20), number of external cells (oogonia and primary oocytes) 12–38 (mode, 20), number of internal cells (spermatogonia and primary and secondary spermatocytes) 5–12 (mode, 8), and number of sperm 8–36 (mode, 14). Diameter of fertilized eggs 9.4 μm; diameter of sperm 1.5 μm.

Infusoriform embryos (Figs 3f, g, 4h–j). Ovoid, rounded to bluntly pointed posteriorly. In full-grown embryos (n=50), length (excluding cilia) 19.7±1.7 μm (mean±S.D.); length-width-height ratio 1: 0.83: 0.78. Cilia at posterior end 4.5 μm long. Refringent bodies present, solid, relatively small, about same size as urn cells, occupying about 25–30% of embryo length when viewed laterally (Fig. 4j). Cilia projecting from ventral internal cells into urn cavity (Fig. 4j). Capsule cells containing many large granules. Full-grown infusoriform embryos (n=50) consisting of 37 cells: 33 somatic and 4 germinal cells. Somatic cells of several types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, and 2 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); external cells without cilia (2 anterior lateral cells, 2 first ventral cells, 2 second ventral cells, and 1 couvercle cell); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, and 4 urn cells). Each urn cell containing 1 germinal cell plus 1 nucleus (Fig. 4j). Nuclei of anterior lateral cells pycnotic. All somatic nuclei becoming pycnotic as infusoriform embryos mature.

Type series. Syntypes: OUM-ME-00004 (1 slide, OUM); SBMNH-358801 (1 slide, SBMNH); No. HF-KA565 (5 slides, author's collection).

Type locality. Japan, Shikoku, Kochi Prefecture, Tosa Bay, off Irino, 32°55′N, 137°10′E.

Host (symbiotype). *Octopus kagoshimensis* (Cephalopoda: Octopoda: Octopodidae), mature female, 55 mm ML, OUM-MO-00003 for KA565.

Site of infection. Within renal sacs; anterior ends (calottes) attached to surface of renal appendages.

Incidence. Dicyemids found in 3 host cephalopods obtained in Tosa Bay (Irino), with 14.3% incidence among 21 cephalopods examined (see Table 1).

Distribution. Known only from the type locality.

Etymology. The specific name refers to Irino, the type locality.

Remarks. *Dicyema irinoense* sp. nov. has been found together with *D. tosaense* in *Octopus kagoshimensis*. It is easily distinguishable from *D. tosaense* based on calotte shape (disc-shaped vs. cone-shaped) and number of peripheral cells of the vermiform stages (22 vs. 16–18).

Dicyema irinoense is very similar to *D. sphyrocephalum* Furuya, 1999, *D. orientale*, and *D. japonicum* in calotte shape, number of peripheral cells in the vermiform stages, and number of cells in infusoriform embryos. However, it differs from *D. sphyrocephalum* in having a stockier body in the full-grown vermiform stages and in having third ventral cells instead of anterior lateral cells in infusoriform embryos (cf. Furuya 1999). It differs from *D. orientale* in the much smaller body size of the full-grown vermiform stages, in having third ventral cells instead of anterior lateral cells, and in having a single nucleus in each urn cell (cf. Furuya and Tsuneki 2004). *Dicyema irinoense* can be distinguished from *D. japonicum* in having a conical calotte at young vermiform stages. In *D. japonicum*, the calotte of vermiform larvae and young vermiform individuals is disc-shaped (Furuya *et al.* 1992a).

Dicyema benthoctopi Hochberg and Short, 1970 also is very similar to *D. irinoense* in both calotte shape and the number of peripheral cells (cf. Hochberg and Short 1970), but the two species are distinguishable based on the number of peripheral cells (always 22 in *D. irinoense* vs. a variable number, 17–23, in *D. benthoctopi*) and the size of the full-grown vermiform stages (larger in *D. benthoctopi*).

Dicyema sphaerocephalum sp. nov.
(Figs 5, 6, Tables 1, 2)

Diagnosis. Medium-sized dicyemids, body lengths typically not exceeding 1100 μm. Peripheral cell number of vermiform stages (i.e., vermiform embryo, nematogen, and rhombogen) 18–20: 4 propolars, 4 metapolars, and 10–12 trunk cells. Calotte spherical. Infusoriform embryos consisting of 37 cells; urn cells with 2 nuclei each.

Description. *Nematogens* (Figs 5a, 6a, c). Body slender; lengths ranging from 500 to 1000 μm, widths from 30 to 70 μm. Peripheral cell number 18–20: 4 propolars, 4 metapolars, 2 parapolars, 6–8 diapolars, and 2 uropolars. Calotte large, spherical. Cilia on calotte short, about 5 μm long, oriented forwards. Cytoplasm of propolar cells stained with hematoxylin, metapolar cells not stained (Fig. 5a, b). Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively. Trunk mostly uniform in width; trunk cells arranged in opposed pairs. Axial cell cylindrical, tapered anteriorly, extending forward to base of propolar cells (Fig. 6c). In axial cell of large individuals, about 10 vermiform embryos present.

Vermiform embryos (Figs 5c, 6e, f). Full-grown vermiform embryos small-sized; lengths ranging from 20 to 50 μm, widths from 8 to 12 μm; peripheral cell number 18–20 (Table 2). Anterior end of calotte bluntly rounded. Trunk cells arranged in opposed pairs. Axial cell tapered anteriorly, sometimes pointed, extending forward to middle of propolar cells (Fig. 5c). Axial cell nucleus typically located in center of

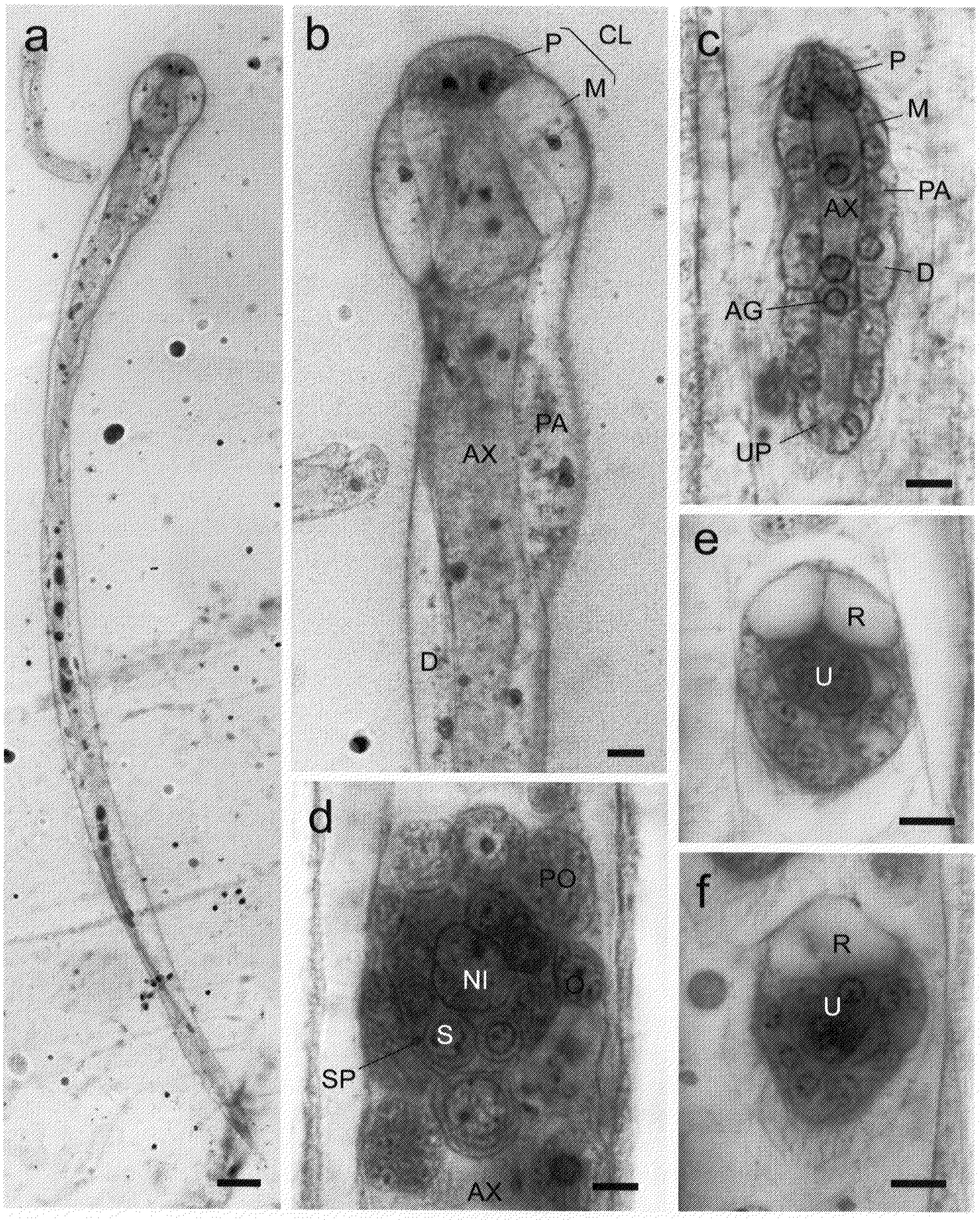

Fig. 5. *Dicyema sphaerocephalum* sp. nov., syntype specimens on slide OUM-ME-00005. a, Nematogen, entire; b, rhombogen, anterior region; c, vermiform embryo within axial cell; d, infusorigen; e–f, infusoriform embryo, horizontal section (e) and sagittal section (f). Scale bars: a=20 μm, b–f=5 μm. Abbreviations as in "Materials and Methods".

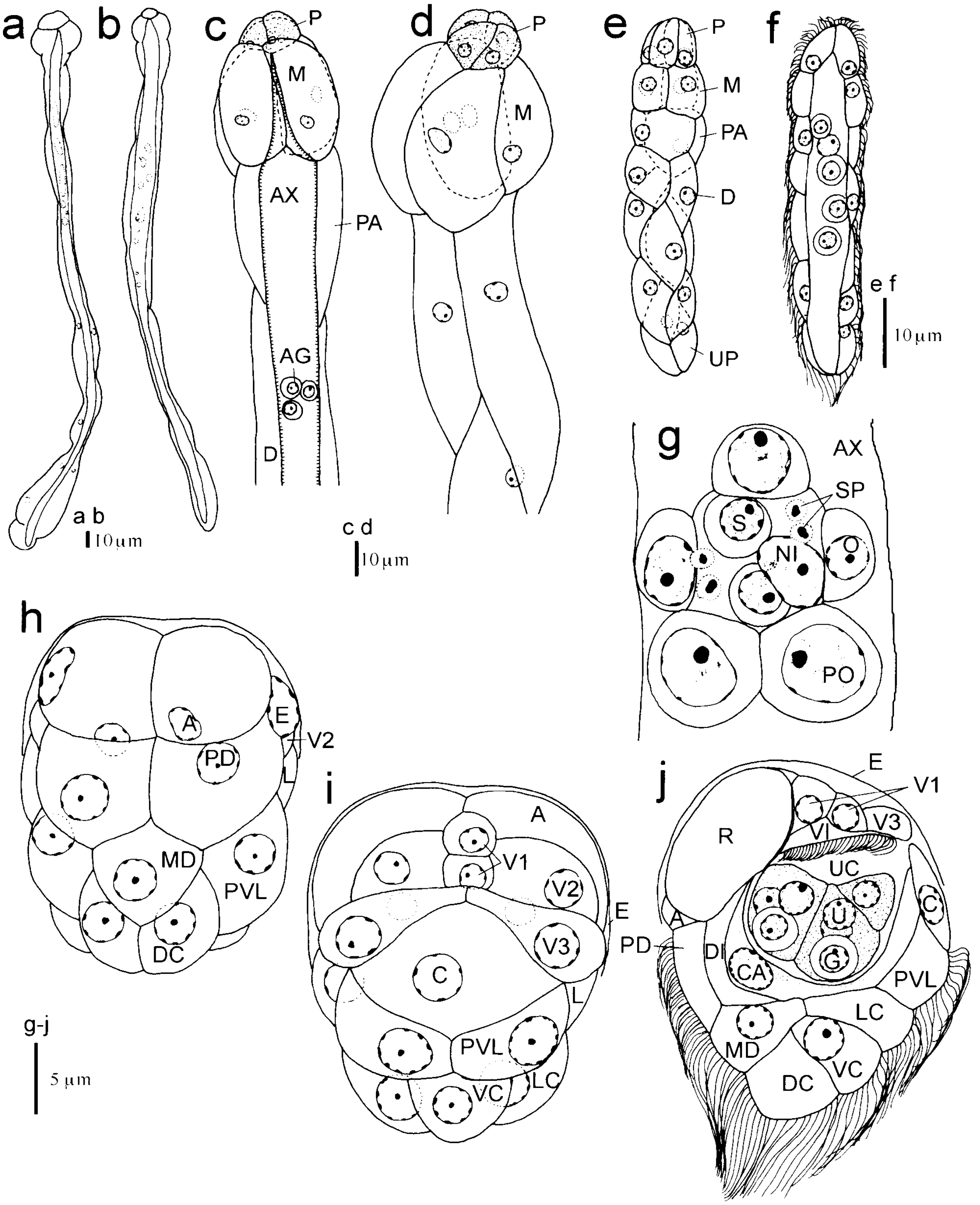

Fig. 6. *Dicyema sphaerocephalum* sp. nov., syntype specimens on slide OUM-ME-00005. a, Nematogen, entire; b, rhombogen, entire; c, nematogen, anterior region; d, rhombogen, anterior region; e–f, vermiform embryos within axial cell, cilia omitted (e) and optical section (f); g, infusorigen; h–j, infusoriform embryos, dorsal view (h; cilia omitted), ventral view (i; cilia omitted), and sagittal section (j). Abbreviations as in "Materials and Methods".

axial cell. Axial cell of full-grown embryos containing 2–4 agametes.

Rhombogens (Figs 5b, 6b, d). Slightly stockier than nematogens, otherwise similar in shape and body proportions; lengths ranging from 500 to 1000 μm, widths from 40 to 70 μm. Peripheral cell number 18–20 (Table 2). Calotte conical as in nematogens. Shape and anterior extent of axial cell similar to those of nematogens. Number of infusorigens present in axial cell 1 or 2; in axial cell of large individuals, 10–15 infusoriform embryos typically present.

Infusorigens (Figs 5d, 6g). Medium-sized. Axial cell of infusorigens usually irregular in shape. In mature infusorigens (n=20), number of external cells (oogonia and primary oocytes) 4–25 (mode, 8), number of internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes) 3–18 (mode, 3), and number of sperm 2–8 (mode, 4). Diameter of fertilized eggs 7.9 μm; diameter of sperm 1.2 μm.

Infusoriform embryos (Figs 5e, f, 6h–j). Ovoid, rounded to bluntly pointed posteriorly. In full-grown embryos (n=50), length (excluding cilia) 20.4±1.8 μm (mean±S.D.); length-width-height ratio 1: 0.82: 0.75. Cilia at posterior end, 5.0 μm long. Refringent bodies present, solid, occupying anterior 40% of embryo length when viewed laterally (Fig. 6j). Cilia projecting from ventral internal cells into urn cavity (Fig. 6j). Cytoplasm of dorsal internal cells transparent. Capsule cells with small granular inclusions. First ventral cells located on median line (Fig. 6i, j). Full-grown infusoriform embryos (n=50) consisting of 37 cells: 33 somatic and 4 germinal cells. Somatic cells of several types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, and 2 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); external cells without cilia (2 first ventral cells, 2 second ventral cells, 2 third ventral cells, and 1 couvercle cell); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, and 4 urn cells). Each urn cell containing 1 germinal cell plus 2 nuclei (Fig. 6j). Nuclei of third ventral cells pycnotic. All somatic nuclei typically becoming pycnotic as infusoriform embryos mature.

Type series. Syntypes: OUM-ME-00005 (1 slide); SBMNH-358802 (1 slide); No. HF-KA686 (5 slides, author's collection).

Type locality. Japan, Shikoku, Kochi Prefecture, Tosa Bay, off Irino, 32°55′N, 137°10′E.

Host (symbiotype). *Octopus kagoshimensis* (Cephalopoda: Octopoda: Octopodidae), mature male, 52 mm ML, OUM-MO-00004 for KA686.

Site of infection. Within renal sacs; anterior ends (calottes) inserted into crypts of renal appendages.

Incidence. Dicyemids found in 6 host cephalopods, obtained in Tosa Bay (Irino), with 28.6% incidence among 21 cephalopods examined (see Table 1).

Distribution. Known only from the type locality.

Etymology. The specific name is composed of two Greek words, *sphaeros*, meaning "sphere", and *cephalos*, meaning "head", in reference to the characteristic spherical calotte of the adult stages.

Remarks. *Dicyema sphaerocephalum* differs from all the other species in the genus principally in its spherical calotte, small propolar cells, swollen metapolar cells, and number of peripheral cells.

Dicyema sphaerocephalum has been found together with *D. tosaense* and *D. irinoense* in *Octopus kagoshimensis*. It is easily distinguished from *D. tosaense* and *D. irinoense* based on the spherical calotte shape and the number of peripheral cells in the vermiform stages.

With regard to the cellular composition and cell number of the infusoriform embryos, *D. sphaerocephalum* is of the typical type for dicyemids (Furuya, Hochberg *et al.* 2004); however, its embryos are exceptional in that the first ventral cells are located on the midline of the embryos.

Discussion

There is considerable taxonomical confusion in the group of *Octopus aegina* (Gray, 1849) which includes *Octopus kagoshimensis* (Robson 1929). Recent reviews suggest that *O. aegina* and *O. kagoshimensis* are distinct and valid species (Norman and Hochberg in press; Huffard and Hochberg in press). Huffard and Hochberg (in press) will propose to place this species-group in the genus *Amphioctopus*.

Octopus kagoshimensis inhabits inshore waters and is widely distributed throughout the Indo-West Pacific from the Indian Ocean to the western and central Pacific (Norman and Hochberg in press). Several species of dicyemid have been found in *Octopus*: *O. aegina* from Taiwan (Furuya *et al.* unpublished), *O. areolatus* from Japan (Furuya and Tsuneki 2003), *O. burryi* (Voss, 1950) from the Gulf of Mexico (Furuya *et al.* 2002), and *O. fangsiao* from Japan (Furuya 1999). Dicyemids were not found in an undescribed *Amphioctopus* from Hawaii that uses a hole or gap in a rock or coral as a living space (Huffard and Hochberg in press). Dicyemids do not appear to infect cephalopods that live in corals and rocks even though they are benthonic in habitat (Furuya, Ota *et al.* 2004).

In this study the three new dicyemid species, *Dicyema tosaense*, *D. irinoense*, and *D. sphaerocephalum*, were found in 18 of 21 examined individuals of *Octopus kagoshimensis* caught in Tosa Bay. The prevalence of dicyemids was 85.7%, which is relatively high. There is a direct relationship between host size and dicyemid occurrence (Furuya *et al.* 1992a): smaller or younger cephalopods of a host species generally do not harbor dicyemids. In *O. kagoshimensis*, however, the three examined individuals that harbored no dicyemids were medium-sized and the absence of dicyemids can not be attributed to host size.

In cephalopods that harbor two or three dicyemid species in the renal sac, the calotte shapes are typically different from each other (Furuya *et al.* 2003a). In *O. kagoshimensis*, two dicyemid species co-occurred in five out of 21 individuals and all three dicyemid species coexisted in the renal sac of one host individual. The calotte shapes of these dicyemid species are different from one another. This is a typical relationship between calotte shape and coexistence pattern.

In *O. kagoshimensis* the most common dicyemid was *Dicyema tosaense*, present in 16 of 21 host individuals examined. When *D. tosaense* and *D. irinoense* co-occurred in the renal sac of a single host individual, *D. tosaense* occupied crypts in the renal tissue whereas *D. irinoense* attached to the surface of the renal appendages. This suggests that niche separation occurs between these dicyemids. Both *D. tosaense* and *D. sphaerocephalum* live in crypts in the renal appendages.

When *D. tosaense* and *D. sphaerocephalum* co-occur in a single host individual, *D. sphaerocephalum* is found less frequently. This species appears to use space where *D. tosaense* is not present. Interspecific interactions might be occurring between *D. tosaense* and *D. sphaerocephalum*.

The size and number of infusorigens are diagnostic characteristics of dicyemid species (Furuya *et al.* 1993). There is a negative curvilinear relationship between the number of infusorigens per rhombogen and the number of gametes per infusorigen (Furuya *et al.* 2003b). Two distinct groups of dicyemid species are apparent. One kind forms a small number of infusorigens and produces a relatively large number of gametes (four to 70) per infusorigen, as in *D. sphaerocephalum* and *D. irinoense*. The other kind tends to produce a large number of infusorigens, each of which has at most 20 gametes per infusorigen, as in *D. orientale*. Rhombogens of *D. tosaense* have a relatively small number of small-sized infusorigens, and thus this species does not belong to either of these two groups. There are, in fact, other exceptional species in which the rhombogen produces small numbers of infusorigens and each infusorigen has a small number of gametes (Furuya *et al.* 2003b). The infusorigens of *D. tosaense* belong to this third group. The body of *D. tosaense* is relatively small, as in other species of this group, and *D. tosaense* probably evolved through progenesis.

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References

Beneden, É. van. 1876. Recherches sur les Dicyémides, survivants actuels d'un embranchement des Mésozoaires. Bulletins de l'Académie Royale de Belgique 42: 3–111.

Couch, J. A. and Short, R. B. 1964. *Dicyema bilobum* sp. n. (Mesozoa: Dicyemidae) from the northern Gulf of Mexico. Journal of Parasitology 50: 641–645.

Furuya, H. 1999. Fourteen new species of dicyemid mesozoans from six Japanese cephalopods, with comments on host specificity. Species Diversity 4: 257–319.

Furuya, H., Damian, R. T. and Hochberg, F. G. 2002. A new species of *Dicyema* (Phylum Dicyemida) from *Octopus burryi* (Mollusca: Cephalopoda) in the Gulf of Mexico. Journal of Parasitology 88: 325–329.

Furuya, H., Hochberg, F. G. and Tsuneki, K. 2003a. Calotte morphology in the phylum Dicyemida: niche separation and convergence. Journal of Zoology 259: 361–373.

Furuya, H., Hochberg, F. G. and Tsuneki, K. 2003b. Reproductive traits of dicyemids. Marine Biology 142: 693–706.

Furuya, H., Hochberg, F. G. and Tsuneki, K. 2004. Cell number and cellular composition in infusoriform larvae of dicyemid mesozoans (Phylum Dicyemida). Zoological Science 21:

877–889.

Furuya, H., Ota, M., Kimura, R. and Tsuneki, K. 2004. The renal organs of cephalopods: a habitat for dicyemids and chromidinids. Journal of Morphology 262: 629–643.

Furuya, H. and Tsuneki, K. 2003. Biology of dicyemid mesozoans. Zoological Science 20: 519–532.

Furuya, H. and Tsuneki, K. 2005. A new species of dicyemid mesozoan (Phylum Dicyemida) from *Sepioteuthis lessoniana* (Mollusca: Cephalopoda), with notes on *Dicyema orientale*. Species Diversity 10: 45–62.

Furuya, H., Tsuneki, K. and Koshida, Y. 1992a. Two new species of the genus *Dicyema* (Mesozoa) from octopuses of Japan with notes on *D. misakiense* and *D. acuticephalum*. Zoological Science 9: 423–437.

Furuya, H., Tsuneki, K. and Koshida, Y. 1992b. Development of the infusoriform embryo of *Dicyema japonicum* (Mesozoa: Dicyemidae). Biological Bulletin 183: 248–257.

Furuya, H., Tsuneki, K. and Koshida, Y. 1993. The development of the hermaphroditic gonad in four species of dicyemid mesozoans. Zoological Science 10: 455–466.

Furuya, H., Tsuneki, K. and Koshida, Y. 1997. Fine structure of a dicyemid mesozoan, *Dicyema acuticephalum*, with special reference to cell junctions. Journal of Morphology 231: 297–305.

Hochberg, F. G. and Short, R. B. 1970. *Dicyemennea littlei* sp. n. and *Dicyema benthoctopi* sp. n.: dicyemid Mesozoa from *Benthoctopus megellanicus*. Transactions of the American Microscopical Society 89: 216–224.

Huffard, C. L. and Hochberg, F. G. in press. Resurrection of the genus *Amphioctopus* (Mollusca: Octopodidae) and description of a new species in the Hawaiian Archipelago. Molluscan Research.

Norman, M. D. and Hochberg, F. G. in press. The current state of octopus taxonomy. Phuket Marine Biological Center Special Publication.

Nouvel, H. 1947. Les Dicyémides. 1re partie: systématique, générations, vermiformes, infusorigène et sexualité. Archives de Biologie 58: 59–220.

Nouvel, H. 1948. Les Dicyémides. 2e partie: infusoriforme, tératologie, spécificité du parasitisme, affinités. Archives de Biologie 59: 147–223.

Nouvel, H. and Nakao, Y. 1938. Dicyémides du Japon. Bulletin de la Société Zoologique de France 63: 72–80.

Robson, G. C. 1929. *A Monograph of the Recent Cephalopoda. Part I. Octopodinae*. British Museum, London, 236 pp.

Short, R. B. 1964. *Dicyema typoides* sp. n. (Mesozoa: Dicyemidae) from the northern Gulf of Mexico. Journal of Parasitology 50: 646–651.

Short, R. B. 1971. Three new species of *Dicyema* (Mesozoa: Dicyemidae) from New Zealand. Antarctic Research Series 17: 231–249.

Short, R. B. and Damian, R. T. 1966. Morphology of the infusoriform larva of *Dicyema aegira* (Mesozoa: Dicyemidae). Journal of Parasitology 52: 746–751.